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RESEARCH ARTICLE

Comparative cardiopulmonary effects of size-fractionated airborne particulate matter

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Abstract

Context: Strong epidemiological evidence exists linking particulate matter (PM) exposures with hospital admissions of individuals for cardiopulmonary symptoms. The PM size is important in influencing the extent of infiltration into the respiratory tract and systemic circulation and directs the differential physiological impacts.

Objective: To investigate the differential effects of the quasi-ultrafine ($PM_{0.2}$), fine ($PM_{0.15\text{-}2.5}$), and coarse PM ($PM_{2.5\text{-}10}$) size fractions on pulmonary and cardiac function.

Methods: Female BALB/c mice were exposed to HEPA-filtered laboratory air or concentrated coarse, fine, or quasi-ultrafine PM using Harvard Ambient Particle Concentrators in conjunction with our nose-only exposure system. These exposures were conducted as part of the "*H*ealth *E*ffects of *A*erosols in *T*oronto (HEAT)" campaign. Following a 4 h exposure, mice underwent assessment of respiratory function and recording of electrocardiograms using the flexiVent® system.

Results: Exposure to coarse and fine PM resulted in a significant reduction in quasistatic compliance of the lung. Baseline total respiratory resistance and maximum responsiveness to methacholine were augmented after coarse PM exposures but were not affected by quasi-ultrafine PM exposures. In contrast, quasi-ultrafine PM alone had a significant effect on heart rate and in reducing heart rate variability.

Conclusion: These findings indicate that coarse and fine PM influence lung function and airways responsiveness, while ultrafine PM can perturb cardiac function. This study supports the hypothesis that coarse and fine PM exerts its predominant physiologic effects at the site of deposition in the airways, whereas ultrafine PM likely crosses the alveolar epithelial barrier into the systemic circulation to affect cardiovascular function.

Keywords: Particulate matter, air pollution, pulmonary function test, respiratory mechanics, airways hyperresponsiveness, heart rate, heart rate variability

Introduction

Epidemiological studies have demonstrated strong association between increasing levels of air pollution and significant adverse health effects resulting in morbidity and mortality (Dockery et al., 1993; Pope et al., 2009; Stieb et al., 2000). In 2007, Toronto Public Health released a report highlighting the significant burden of illness associated with air pollution (Toronto Public Health, 2007).

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Abbreviations

BALF, bronchoalveolar lavage fluid
bpm, beats per minute
CAP, concentrated ambient particles
Cst, quasistatic compliance
ECG, Electrocardiogram
Est, quasistatic elastance
HEPA, high efficiency particulate air
HRV, heart rate variability
PBS, phosphate buffered saline
PM, particulate matter
PV, pressure–volume
RR, interval, the duration from one QRS complex to the next
Rrs, resistance of the total respiratory system
SDNN, standard deviation of normal to normal sinus beat interval

The report estimated that traffic-related air pollution is responsible for about 400 premature deaths and 1,700 hospitalizations annually in Toronto (Toronto Public Health, 2007). In 2008, the Canadian Medical Association similarly estimated that 21,000 deaths occur annually due to air pollution, of which 2,682 were as a result of acute short-term exposures (Canadian Medical Association, 2008). Although much of the epidemiological evidence indicates a small relative risk associated with air pollution, these reports suggest that a substantial public health burden can result in large population. Airborne particles have been extensively implicated as causative in this increased cardiopulmonary burden (Schlesinger et al., 2006). Acute increases in particulate matter (PM) have been associated with worsening of respiratory symptoms in asthmatic children and in adults with chronic obstructive pulmonary disease (Brauer et al., 2002; Brunekreef & Forsberg, 2005; Liu et al., 2009; Viera et al., 2009). Exposure to elevated PM concentrations has also been associated with increased risk for cardiac arrhythmia, myocardial infarction, and congestive heart failure (Belleudi et al., 2010; Peters et al., 2000; Peters et al., 2001; Schwartz & Morris, 1995). In addition to the substantial epidemiological evidence, a growing number of controlled human exposure and experimental animal studies have confirmed the negative effects of ambient PM (Brook et al., 2002; Brook et al., 2009; Graff et al., 2009; Liao et al., 2010; Lippmann & Chen, 2009; Samet et al., 2009; Sivagangabalan et al., 2011; Tong et al., 2010; Urch et al., 2004; Urch et al., 2005). The majority of these adverse effects have been identified in population with pre-existing cardiopulmonary disease or in animal models, which represent segments of these susceptible populations (He et al., 2010; Quan et al., 2010; Rohr et al., 2010).

Although there has been increased recognition of the potential for PM-associated toxicity, the linkages between specific physiochemical properties of PM and observed health effects remain to be determined. A number of physicochemical properties have been investigated for their potential in generating adverse biological responses. For regulatory purposes and toxicological evaluations, particle size is considered an important factor in influencing PM toxicity (Schlesinger et al., 2006). PM is generally categorized into three size fractions: Coarse (aerodynamic diameter (AD) 2.5 to 10 μm), Fine (AD < 2.5 μm), and Ultrafine (AD: <0.1 μm). The size of the PM has been recognized as an important determinant of the associated health risk as it influences the extent of infiltration into the respiratory system and systemic circulation (Lippmann & Chen, 2009; Oberdorster et al., 1994). Coarse PM deposits along the conductive airways of the respiratory tract, while fine and ultrafine PM are inhaled deeper into the lungs penetrating as far as the alveolar gas-exchange regions (Lippmann & Chen, 2009; Oberdorster et al., 1994). Furthermore, small-sized PM fractions or specific components of PM may cross the alveolar barrier and enter into the systemic circulation (Elder & Oberdorster, 2006; Lippmann & Chen, 2009; Wallenborn et al., 2007). The different-sized particles exhibit characteristic deposition profiles that may partially explain the differential health effects observed. For instance, numerous epidemiological studies have documented an increased risk for cardiovascular events specifically with exposure to the fine PM fraction (Belleudi et al., 2010). On the other hand, PM composition has also been associated with toxicity. Source-specific PM, which can have widespread differences in organic, inorganic, soluble, and insoluble PM components, has been linked to differential biological responses and health outcomes (Akhtar et al., 2010; Schlesinger et al., 2006; Wallenborn et al., 2007). Particle size and chemical composition are both important modulators of toxicity, and thus, the observed toxicity may relate to a combination of these factors.

In this study, a comparative analysis of the physiologic effects of the three PM size fractions is performed, evaluating both pulmonary and cardiovascular endpoints to gain deeper insight on the extent of the role of PM size in influencing toxicity. Changes in pulmonary mechanics, heart rate (HR), and heart rate variability (HRV) following inhalation of concentrated ambient PM are reported. In addition, the chemical constituents of the PM are characterized to investigate any possible linkages with cardiopulmonary response. Most importantly, the health effects of acute PM exposure in naïve mice are investigated to understand the potential health risks associated with PM exposures in a normal, otherwise healthy population.

Materials and methods

Animals and exposure regimen

Animals were treated humanely and with regard to alleviation of any suffering in accordance with the guidelines

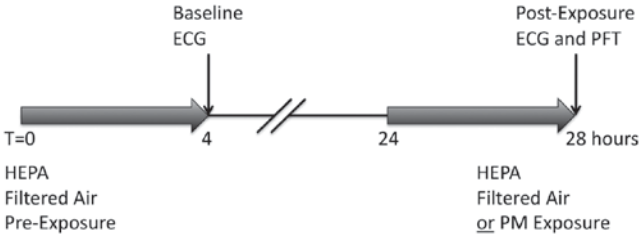

Figure 1. Schematic diagram representing the timeline of the outcome measurements for the 28-hour exposure protocol.

outlined in the Canadian Council of Animal Care. The protocol was approved by the University of Toronto Faculty Advisory Committee on Animal Services. Normal control female BALB/c mice (Charles River Laboratories, Saint Constant, PQ), 6–8 weeks of age, and weighing an average of 18 g (range: 16–21 g) were used for this study. The mice were divided into four groups: filtered air (FA), coarse PM, fine PM, and quasi-ultrafine PM. All mice underwent a baseline 4 h pre-exposure to HEPA-FA, 24 h prior to the actual control HEPA-FA or PM exposure. The pre-exposures were conducted in a modified inExpose nose-only inhalation system (SciReq Inc., Montréal, PQ) contained within a Plexiglas chamber, as described previously (North et al., 2011), which allows for the exposure of six mice at a time. Immediately after the pre-exposure, mice were anesthetized with a mixture of ketamine (50 mg/kg i.p., Bioniche, Belleville, Ontario, Canada) and xylazine (10 mg/kg i.p., Bayer Inc., Toronto, Ontario, Canada), and baseline electrocardiograms (ECGs) were obtained. About 24 h later, the mice were exposed to one of the three different PM size fractions (coarse, fine, or quasi-ultrafine) or FA for 4 h at a flow rate of 2 L/min (Figure 1). Immediately after the exposures, mice were anesthetized and ECGs and pulmonary function tests were conducted, as described below.

Measurement of gaseous species and meteorological parameters

Data for the ambient gaseous pollutant concentrations (SO_2, O_3, NO_2, NO, and CO) were obtained from an Ontario Ministry of the Environment fixed-site air-monitoring station located in downtown Toronto. Hourly temperature and humidity data were provided by Environment Canada's fixed-site air-monitoring station located at Pearson International Airport in Toronto.

PM exposure system and characterization

Mice were exposed to concentrated ambient PM (CAPs) using the Southern Ontario Centre for Atmospheric Aerosol Research (SOCAAR) Concentrated Ambient Particulate Exposure Facility (CAPEF) located within the Gage Occupational and Environmental Health Unit in Toronto. Ambient air was drawn in from a busy downtown street. The concentrator, exposure system, and site have been previously described (North et al., 2011; Sivagangabalan et al., 2011; Urch et al., 2004; Urch et al., 2005). Briefly, coarse and fine CAPs were concentrated using high volume, multistage virtual impactor systems. In the ultrafine concentrator, ambient aerosols were drawn sequentially through a saturator and condenser. This allowed for ultrafine particles to grow to supermicrometer sizes through condensation of water. The grown particles were further drawn through a two-stage virtual impactor, with an upper cutpoint of 1 μm. After concentration enrichment, the original size distribution of ambient ultrafine particles was restored in a thermal–dilution dryer, and particles larger than 0.2 μm were then removed by inertial impaction in a size selective outlet (Gupta et al., 2004).

The exposures were conducted as part of an intensive collaborative campaign (the Health Effects of Aerosols in Toronto [HEAT] Campaign) that took place from February 19, 2010 to March 19, 2010. The objectives of the campaign were to characterize the performance of the concentrators and to conduct simultaneous *in vitro* and *in vivo* studies evaluating biological/health effects of size-fractionated airborne PM in relation to its physicochemical properties. Mouse exposures were conducted in the morning (08:00–12:00) between March 1 and March 19, 2010. Additional fine and ultrafine exposures were conducted on April 9 and April 23, 2010, respectively. During the exposures, PM filter samples were collected at an airflow rate of 2 L/min for gravimetric determination of total PM mass. Briefly, chemical species were analyzed in acid extracts of filter samples using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES, Perkin Elmer Optima 3700 DV) in axial mode, at the Analytical Laboratory for Environmental Science Research and Training (ANALEST) facility of University of Toronto. Concentrations of organic carbon (OC) and elemental carbon (EC) were determined using a Sunset EC–OC analyzer according to the National Institute of Occupational Health and Safety (NIOSH) 5040 protocol. The anions and cations were assayed with ion chromatography (Dionex ICS-2000). The black carbon (BC) concentration was measured semi-continuously with a 1 s frequency using a photo-acoustic soot spectrometer (PASS, Droplet Measurement Technology, USA). A photo-electric aerosol sensor (PAS, Model PAS2000CE, EcoChem Analytics) was used for semi-continuous determination of particulate-bound polycyclic aromatic hydrocarbons (p-PAHs) with a 1 min integration time. The BC and p-PAH concentrations were integrated over the time of exposure.

Pulmonary function evaluation

Following the PM or FA exposures, mice were anesthetized with ketamine and xylazine (i.p.) and then intubated with an 18-gauge stainless steel cannula (BD Biosciences Canada, Mississauga, Ontario, Canada). The mice were then attached to the flexiVent® system (SciReq Inc., Montreal, QC) for *in vivo*, ventilator-based assessment of respiratory mechanics (Ilies et al., 2010; North et al., 2009; North et al., 2011). Mice were

ventilated at 150 breaths per minute, with a tidal volume of 10 mL/kg and a positive end expiratory pressure (PEEP) of 3 cm H_2O. After intubation, the lungs were inflated to 30 cm H_2O to standardize the volume history and provide an estimate of the total lung capacity. Pressure–volume (PV) loops were also performed to determine underlying respiratory mechanics (i.e., quasistatic compliance [C_{st}] and quasistatic elastance [E_{st}]). Baseline measurements were collected after a 15 min acclimation period. Respiratory tone was assessed using the linear first-order single compartment model, which uses forced oscillation to calculate resistance of the total respiratory system (R_{rs}), as well as compliance and elastance. Following acquisition of baseline values, airways responsiveness was determined by administering increasing concentrations of methacholine (0.1–100 mg/mL in PBS) by nebulization directly into the ventilatory circuit, synchronized with inspiration. All data points were collected using the flexiVent® software and analyzed offline using Excel (Microsoft, Redmond, WA, USA).

Cardiovascular function evaluation and analysis

The HR and ECGs were acquired using the flexiVent® system. Baseline and post-exposure HR and ECG recordings were both conducted in anesthetized mice (i.p. ketamine/xylazine, as above). Electrodes were placed cutaneously in a Lead III configuration (left leg, left arm, right leg). An electrode gel (Signa gel, Parker Laboratories Inc., Fairfield, NJ, USA) was used to minimize skin-electrode resistance. All data were collected using the flexiVent® software and analyzed offline using Prism 4.0c (GraphPad Software, San Diego, CA, USA). Ten consecutive ECG Snapshot data sets (~35–45 consecutive beats), which were collected prior to methacholine administration, were used for subsequent offline ECG analysis. The ECG data sets were graphed in Prism, the peaks identified by analyzing the area under the curve, and HR was determined based on the duration of the RR interval. Variations in the normal RR intervals, or HRV, were analyzed using the time-domain parameter standard deviation of normal to normal sinus beat intervals (SDNN) (Zareba et al., 2001).

Bronchoalveolar lavage and total cell count

Following pulmonary function testing, bronchoalveolar lavage was performed in a subset of mice and total and differential bronchoalveolar lavage fluid (BALF) cell counts were determined, as described previously (North et al., 2011). Differentials were performed on a subset of the BALF samples.

Statistical data analyses

All values are expressed as the mean ± standard error. Mann–Whitney *t*-tests or one-way analysis of variance (ANOVA) with Kruskal–Wallis test were used for binary and multiple comparisons, respectively, between the groups. Differences were considered significant when $p < 0.05$. Statistical analyses were performed using Prism 4.0c (GraphPad Software Inc., La Jolla, CA, USA).

Results

Exposure characterization

Coarse, fine, and ultrafine PM exposures were achieved with 4 h average mass concentrations of 793, 254, and 401 μg/m³, respectively. The coarse PM total mass exposures were different on the two exposure days, with a mass concentration of 1,284 μg/m³ on day 1 and 302 μg/m³ on day 2. The fine PM exposure total mass ranged from 162 to 432 μg/m³. The total mass of the ultrafine PM exposures ranged from 237 to 535 μg/m³. The mass concentration of each 4 h exposure, ambient PM, gas, and weather concentrations are summarized in Supplementary Table S1. The chemical compositions (i.e., metals, EC, organic matter, and water soluble ions) of ambient coarse, fine, and ultrafine PM collected during the campaign are presented in Figure 2. While coarse PM was predominantly composed of water-soluble ions and metals, the composition of fine

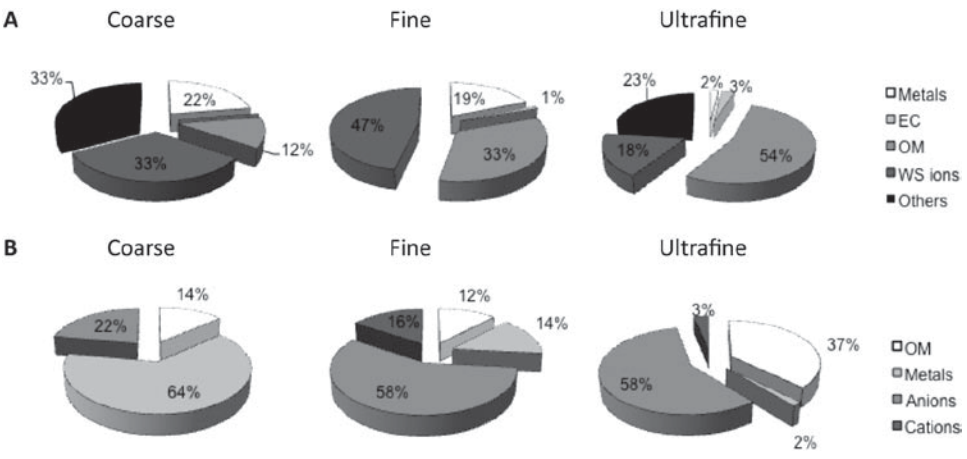

Figure 2. Chemical composition of total (**A**) and water-soluble (**B**) concentrated coarse, fine, and ultrafine PM. EC = elemental carbon, OM = organic mass, WS = water-soluble. Anions = NO_3^-, SO_4^{2-}, $C_2O_4^-$, PO_4^{3-}, Cl^-, Cations = Na^+, K^+, NH_4^+.

PM was predominantly water-soluble ions and organic matter and more than half of the ultrafine PM was comprised of organic matter. Concentrations of the specific elemental constituents in the three PM size fractions are listed in Supplementary Table S2.

Effect of PM on baseline resistance and airways responsiveness

Coarse PM exposure induced a statistically significant ($p<0.05$) increase in baseline respiratory resistance compared with FA controls (Figure 3A). Mice exposed to fine PM exhibited a slight, but not significant, increase in baseline resistance compared with FA control. Ultrafine PM exposure did not result in change in baseline resistance. Meanwhile, the maximum response to methacholine was significantly augmented (1.4-fold increase) in normal control mice exposed to coarse PM, while no significant changes were observed following exposures to fine or ultrafine PM compared with FA controls (Figure 3B).

To further investigate the contribution of PM mass concentration to changes in respiratory system responsiveness, exposures were stratified into "low" and "high" levels, based on a cut-off point of 350 μg/m³, that is, exposure to concentrations of less than 350 μg/m³ were considered as relatively "low" exposures and those greater were considered "high" exposures for all PM groups. Acute episodic increases in PM concentrations have been noted to occasionally exceed 350 μg/m³ in some regions, as discussed below. Based upon this stratification, it is noted that the maximum responsiveness to methacholine was significantly augmented following exposures to high mass concentrations of both coarse and fine PM (Supplementary Figure S1). Ultrafine PM did not exhibit any effect on maximum airways responsiveness. There were no significant differences observed between the low and high within each CAP type.

Effect of PM exposures on respiratory function

The PV loops were used to assess the mechanical properties of the respiratory system (Supplementary Figure S2 and Supplementary Table S3) and to determine the C_{st} and E_{st}. A significant decrease in C_{st} was observed following coarse PM exposures (Figure 4A); similarly, E_{st} was significantly augmented after coarse and fine exposures

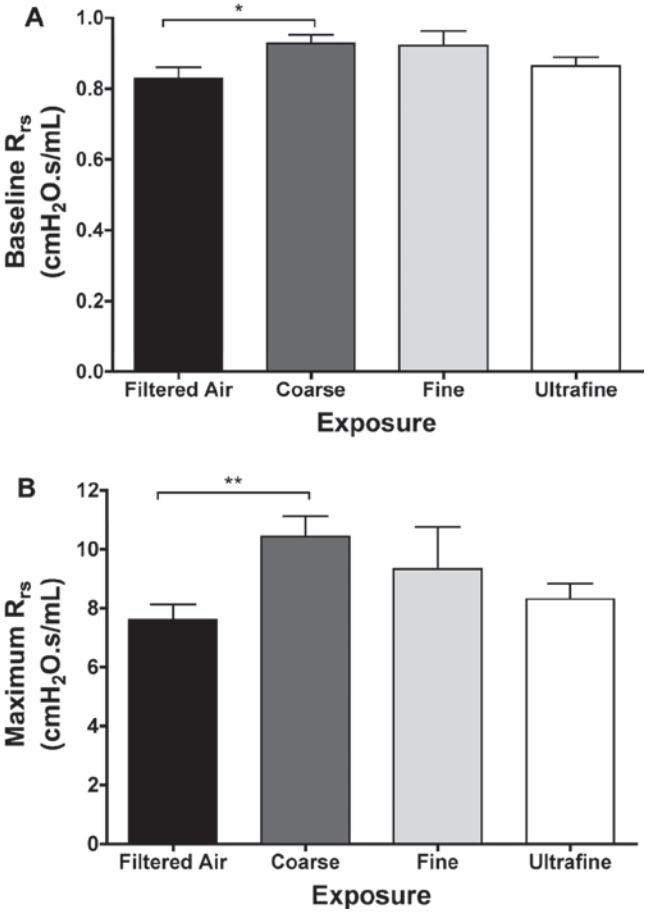

Figure 3. Effect of PM exposures on methacholine responsiveness. (A) Baseline total respiratory resistance (R_{rs}) and (B) maximum response ($R_{rs\ max}$) to methacholine in normal mice exposed to the three different size fractions of PM or FA. Data are expressed as the mean ± standard error. *$p<0.05$, **$p<0.01$; $n=9$–14 mice/group.

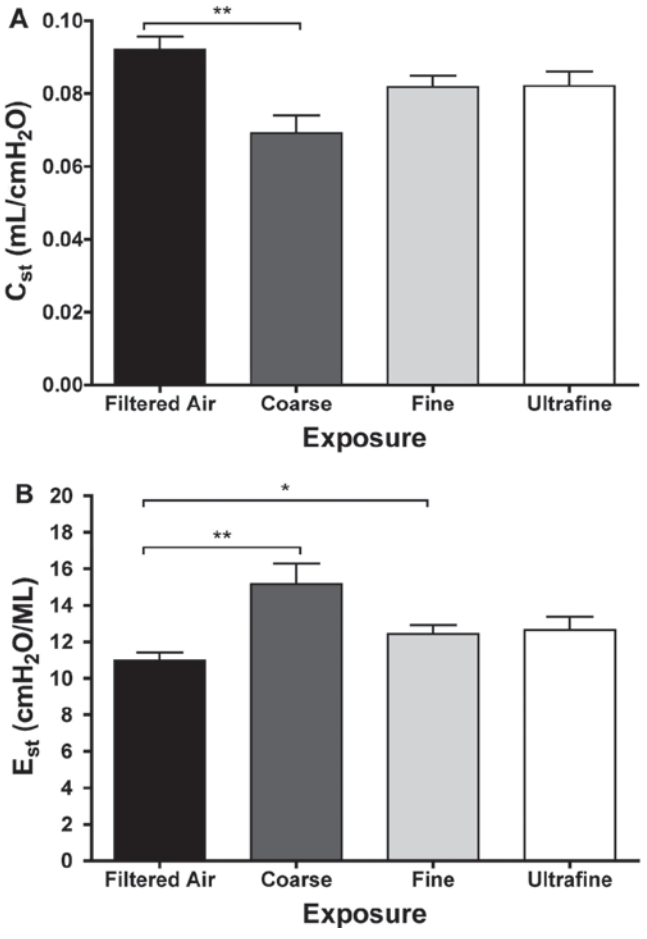

Figure 4. Effects of different PM exposures on respiratory mechanics. (A) Quasistatic compliance (C_{st}) was significantly decreased with coarse PM exposures, while (B) Quasistatic elastance (E_{st}) was significantly augmented in coarse and fine exposures compared with FA controls. Data are expressed as the mean ± standard error. *$p<0.05$, **$p<0.01$; $n=9$–14 mice per group.

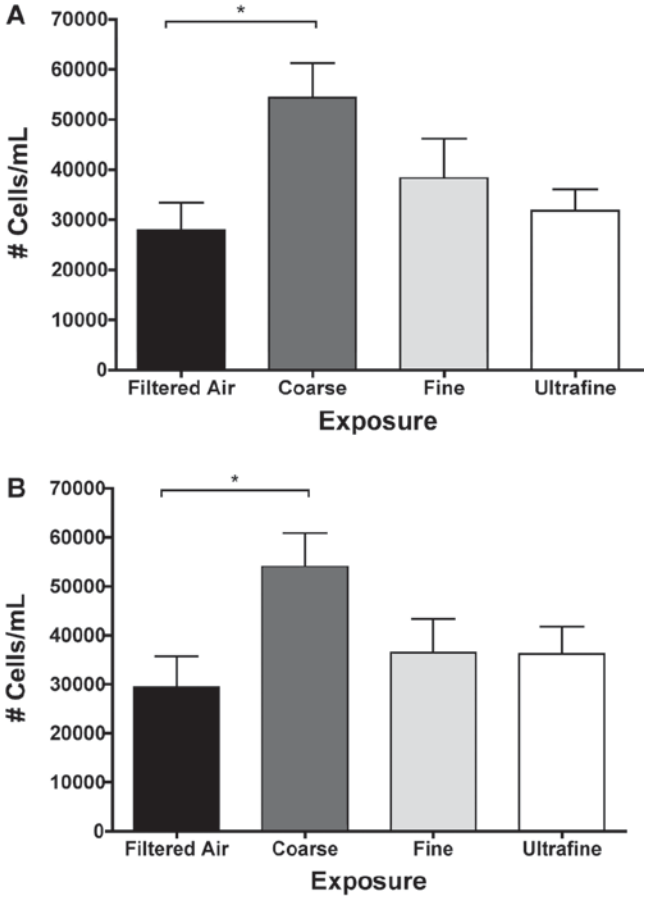

Figure 5. Effect of different PM exposures on BAL cell counts. (A) Total cell count in BALF from PM-exposed mice and FA controls (n = 6–10 mice per group). (B) Increased numbers of macrophages were observed in the coarse PM-exposed mice compared with FA controls. *$p < 0.05$; n = 5–7 mice per group. Data are expressed as the mean ± standard error.

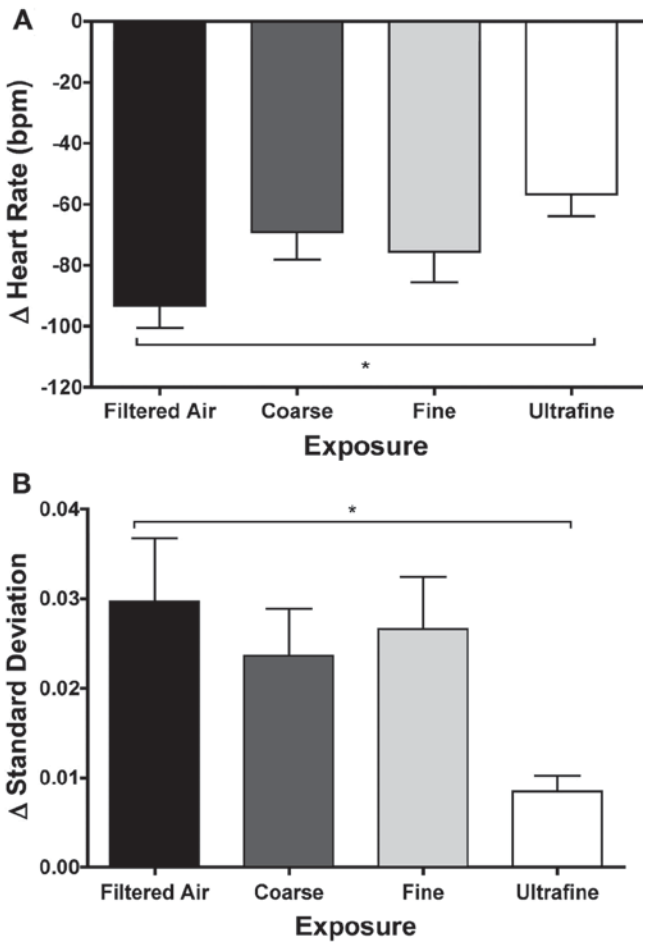

Figure 6. Cardiovascular effects of different PM sized fractions. (A) The change in HR after PM exposure was significantly altered with ultrafine exposures. (B) Variability of normal-to-normal (RR) peak intervals from ECG recordings was significantly attenuated with ultrafine exposures. Data are expressed as the mean ± standard error. *$p < 0.05$, **$p < 0.01$; n = 9–14.

compared with FA controls (Figure 4B). No significant changes in either C_{st} or E_{st} were observed after ultrafine PM exposure.

Inflammatory responses in BALF total cell count

Coarse PM exposure resulted in a significant enhancement (more than 2-fold increase) of the total number of cells in the BAL samples compared with FA controls (Figure 5A). Fine and ultrafine PM exposures did not induce significant changes in the BALF total cell counts versus FA. The differential cell counts demonstrated a statistically significant increase in the number of macrophages in coarse PM-exposed mice compared with FA (Figure 5B). While few neutrophils were observed, there was a slight, albeit statistically insignificant increase in neutrophil counts in BALF from fine PM exposed mice versus FA (data not shown).

Effect of different PM exposures on HR and HRV

The average HR for mice during the pre-exposure was 187 ± 4 bpm. The HR on the exposure day was significantly reduced in all mice, including FA controls compared with the pre-exposure baseline, suggesting that the mice were somewhat habituated to the nose-only exposure system. However, the ultrafine PM exposures showed the largest difference in change in HR compared with FA controls ($p < 0.05$) (Figure 6A). Change in HRV, as assessed by the SDNN, was also significantly decreased (three-fold), following ultrafine PM exposure (Figure 6B) ($p < 0.05$ to both pre exposure and FA controls). There were no significant changes in HR and HRV parameters following either coarse or fine exposures.

The contribution of particle mass concentration to the HRV response by stratification of the exposures into "low" and "high" concentrations was assessed. Both low and high concentrations of ultrafine PM exposure decreased HRV compared with FA controls (Supplementary Figure S3). Interestingly, low concentrations of coarse PM exposure also resulted in a significant reduction of HRV.

Discussion

There is compelling epidemiological evidence that links increasing particulate air pollution levels with adverse

cardiopulmonary health outcomes in the population (Brunekreef & Forsberg, 2005; Liu et al., 2009; Peters et al., 2000; Peters et al., 2001). Recently, a number of studies involving controlled exposures in both humans and animals have also demonstrated some of the adverse health effects associated with PM (Brook et al., 2002; Brook et al., 2009; Lippmann & Chen, 2009; North et al., 2011; Sivagangabalan et al., 2011; Urch et al., 2004; Urch et al., 2005). The PM is a complex mixture of constituents formed through both natural and anthropogenic processes that can vary significantly in chemical composition between locations within and across distinct geographic and climatic regions. However, despite the diverse array of types and sources of PM, effects on cardiopulmonary health have been consistently identified. To better understand the specific circumstances that lead to the various health outcomes and provide a means for improved regulation of PM, one of the major goals of recent studies has been to determine the relationship between specific physicochemical properties of PM and their corresponding health effects.

The present study specifically compared the effects of PM size on both pulmonary and cardiovascular functional endpoints in normal control mice. There are only a few studies that have previously shown pulmonary effects of PM in normal mice (Cho et al., 2009; Happo et al., 2010; Wang et al., 2008); however, these studies utilized intratracheal instillation or pharyngeal aspiration for PM exposures. The PM administration in this manner could result in significantly higher effective doses (Brain et al., 1976). Furthermore, as the site of deposition is independent of particle size with intratracheal instillation (Brain et al., 1976), this approach could not be used to test the hypothesis that differential health effects occur based on the predicted site of size deposition. In the present study, a nose-only system was used to expose the mice to concentrated ambient particles (CAP), which are considered to be much more reflective of real PM constituent mixtures and the direct inhalational route better reflects real-life exposure conditions.

Changes in respiratory airways resistance and airways hyperresponsiveness following PM exposures have been previously observed in several human and animal studies (McCreanor et al., 2007; Nordenhall et al., 2001; North et al., 2011; Stenfors et al., 2004). These changes have been reported more frequently in studies on patients with asthma or COPD, as well as in animals with allergically inflamed airways (Gong et al., 2003; Holgate et al., 2003; Nordenhall et al., 2001; North et al., 2011). Various measurement indices, such as the enhanced pause (Penh) and the airways pressure time index (APTI) have been used in other animal studies to suggest changes in airways responsiveness (Cho et al., 2009; Laks et al., 2008; Wang et al., 2008). The trend clearly suggests that there is an increase in airways resistance and responsiveness following PM exposure, specifically with the coarse and fine PM size fractions. These studies, as mentioned above, used intratracheal instillation as their method of PM administration. The study, using the nose-only exposure system, similarly demonstrated significant increase in total airways resistance and responsiveness to methacholine in normal control BALB/c mice following coarse PM exposure; methacholine responsiveness is an important parameter to consider as methacholine challenge is used to clinically diagnose airways diseases, such as asthma and COPD. Interestingly, there also appeared to be a dose-dependent effect of PM, with higher concentrations of both coarse and fine PM leading to greater pulmonary responses. However, while this study used reasonably large sample sizes for statistical comparisons, the number of exposure days was limited to 2–3 independent days for each PM size fraction. Thus, future studies should explore this dose response further over a wider range of concentrations, particularly at the lower end of the dose range.

It was demonstrated that PM has the potential to induce changes in respiratory mechanics following acute exposures. The PV curves shift downwards with coarse and fine PM exposure, along with a corresponding decrease in compliance and increase in elastance, which are similar to the changes in respiratory mechanics observed with obstructive lung disease. These findings are comparable with a previous study that investigated respiratory mechanics following diesel PM exposure (Laks et al., 2008), and demonstrated an increase in static elastance following intratracheal instillation of diesel particles in male BALB/c mice (Laks et al., 2008). Furthermore, a recent study by Wang et al. (2011) reported a dose-dependent increase in E_{st} following exposure to multi-walled carbon nanotubes. The effects of PM exposure on respiratory mechanics in mice are not well established; at this point, there are only a handful of studies that demonstrate findings on respiratory mechanics. Further studies should be conducted to confirm the role of both acute and chronic PM exposures on respiratory mechanics.

Airways inflammation, characterized by enhanced numbers of leukocytes in BAL samples, has also been commonly observed following PM exposures. In healthy C57BL/6J mice exposed to PM collected from six European cities, total cell counts in BALF increased following both single and multiple dosing of coarse PM, while total cell count increased only after multiple dosing episodes of fine PM (Happo et al., 2010). Similarly, mice exposed to coarse PM collected "close to the road" and "away from the road" also exhibited increased total cells in the BALF (Cho et al., 2009). There was no significant difference in effect between sampling location for coarse PM. This increase in total BALF cell count is primarily attributed to increases in polymorphonuclear cells, that is, neutrophils (Brito et al., 2010; Cho et al., 2009; Happo et al., 2010; Laks et al., 2008; Tong et al., 2010). While a small increase in neutrophil counts with fine PM exposure was observed, this study instead demonstrated that there was a primary increase in the macrophage

population following coarse PM exposure. In general, toxicological studies evaluating comparative effects of the three size fractions have usually presented coarse PM as more potent in inducing pulmonary responses relative to fine and ultrafine PM exposures (Cho et al., 2009; Happo et al., 2010; Tong et al., 2010). Biological and crustal materials are more commonly associated with the coarse PM fraction (Schlesinger et al., 2006) and inflammatory responses may be the consequence of a combination of both the infiltration and retention of large particles that are not easily cleared, as well as the potent biogenic components.

The PM-associated increase in cardiovascular morbidity and mortality has been hypothesized to occur through changes in autonomic control of the heart (Pham et al., 2009; Zareba et al., 2001). The HR and HRV analyses provide quantitative insight into the dynamics of the sympathetic and parasympathetic factors influencing the autonomic nervous system (Zareba et al., 2001). It is observed that the change in HR to be most significantly altered following ultrafine exposures. Some studies have suggested that PM-induced changes were consistent with increased sympathetic nervous system activity (Brito et al., 2010). While there may have been an increase in HR following ultrafine PM exposure, the results are difficult to interpret as HR was decreased following all PM exposures, including the controls, likely due to the stress of the initial HEPA-FA exposure and/or habituation to the pre-exposure conditions.

In general, the majority of human and animal studies have reported a decrease in HRV following PM exposures (Brook et al., 2009; Chuang et al., 2005; Pham et al., 2009; Weichenthal et al., 2011). Reduced HRV has been associated with increased risk of cardiac dysfunction and cardiac-related mortality (La Rovere et al., 2003; Lombardi et al., 2001; Tsuji et al., 1996). Studies of the changes in HRV induced by PM have been somewhat inconsistent among the three size fractions. Many epidemiological studies have repeatedly linked fine PM exposure with decreased HRV, while no major associations have been made with coarse PM exposures. On the other hand, human and animal studies have shown HRV changes with all three size fractions (Chen et al., 2010; Graff et al., 2009; Pham et al., 2009; Samet et al., 2009). This study demonstrated reduced HRV only in response to the ultrafine PM exposures; the ultrafine PM mass concentration did not significantly affect the magnitude of the response. In a similar comparative study looking at effects of oropharyngeally instilled PM in mice, pulmonary effects were observed following coarse and fine PM exposures and cardiac changes were exclusively observed with the ultrafine size fraction (Tong et al., 2010). Consistent with our findings, another comparative study looking at the effect of the three size fractions in cardiac and hypertensive patients also noted reductions in SDNN and another HRV parameter, r-MSSD (root mean square of successive differences in NN intervals) exclusively with the PM 0.3–1.0 μm fraction and not with the larger ($PM_{1.0–2.5}$ and $PM_{2.5–10}$) PM size fractions (Chuang et al., 2005). Furthermore, in a recent study of healthy participants in Ottawa, Canada, HRV was found to be reduced following acute exposure to traffic-induced ultrafine particles during exercise (Weichenthal et al., 2011). Thus, further investigation of the differential cardiac effects of the PM size fractions will be necessary in future.

Stratifying our exposures into "low" and "high" concentrations revealed that lower exposures to coarse PM also resulted in a significant reduction in HRV. The chemical composition data does not suggest any obvious difference for any specific constituent that would be responsible for this effect; however, this is difficult to assess as this study only involved two separate coarse PM exposures. The "low" coarse PM concentration day also coincided with the only exposure day that was carried out in the presence of significant rain (increased relative humidity), thus reducing the ambient coarse PM levels and in turn the CAP exposure levels. Higher concentrations of fine PM also elicited a small, but not significant, reduction in HRV. Some animal studies have shown a reduction in HRV following fine PM exposure but these studies have been performed in various animal models of disease and HRV data collection periods were much longer; ranging from 6–48 h for acute exposures to months for chronic exposures (Chen et al., 2010; Huang et al., 2010; Pham et al., 2009). This paper highlights the association between ultrafine PM exposure and changes in HRV. However, there is evidence for cardiovascular effects following exposure to gaseous pollutants. Our ambient pollution data (Table S1) indicates that there was some variability in the levels of nitrogen oxides between the exposure days. Increases in ambient NO_2 concentrations have been shown to be inversely associated with the standard deviation of normal to normal interval, time domain analysis of HRV in humans (Chan et al., 2005; Weichenthal et al., 2011). However, due to the small number of exposures for each PM size fraction, the impact of the ambient gases or relative humidity on the mouse exposures within the chamber or on the physiological outcomes were not directly investigated. Future investigations should be performed to dissect the effects of ambient gaseous exposures from particles.

The ambient gas and PM concentrations during the current exposures were consistent with previous studies performed at this urban setting (North et al., 2011; Urch et al., 2004; Urch et al., 2005). The CAP chemical constituent concentrations were greater with this animal study compared to the controlled human exposure studies, as higher total PM (CAP) concentrations were targeted but were similar proportionally (Brook et al., 2002; Brook et al., 2009; Sivagangabalan et al., 2011; Thompson et al., 2010; Urch et al., 2004; Urch et al., 2005). Except for the one high coarse PM exposure day, the CAP concentrations of all other exposure days were not unrealistically high for animal studies (Harkema et al., 2009; North et al.,

2011). These concentrations are much higher than the 7–15 μg/m^3 average $PM_{2.5}$ concentrations in Canadian cities (Jeong et al., 2011) but concentrations ranging from 100–500 μg/m^3 concentrations have been recorded as acute 1 h to 4 h episodes of ambient PM in some highly populated urban metropolises (Bathmanabhan & Madanayak, 2010; Cheng & Li, 2010; Pérez et al., 2000; Zhao et al., 2009). Chemical constituents of PM, such as OC, carbonic, and specific transition metals have been correlated with various cardiopulmonary outcomes in previous studies (Urch et al., 2004); however, the limited number of exposures for each size fraction in this study makes it difficult to assess the role of specific PM constituents. Furthermore, evaluating the effects of PM size is not independent of chemical composition as certain chemical constituents were primarily confined to specific PM size fractions. Future investigations will be needed to increase the number of exposures and better evaluate the cardiopulmonary response over a wider range of PM concentrations, as well as size-specific constituents that may be responsible for the observed cardiopulmonary effects. Further, while some gender differences in the effects of PM on cardiovascular and pulmonary function/development have been reported in epidemiological studies (Clougherty, 2010; Dong et al., 2011; Gehring et al., 2002; Nuvolone et al., 2011; Zhang et al., 2011), at present there is no consensus on underlying mechanisms (Clougherty, 2010; EPA, 2009). However, one recent report has demonstrated a reduction in arteriolar responsiveness in females following elevated ambient fine particulate exposures (Pope et al., 2011). One suggested mechanism related to gender-based differences relates to the structure of the airways in males versus females where airways are larger and resistance lower in infant females compared with males (Gehring et al., 2002). Thus, future investigations will need to determine whether these potential gender-based differences in the adverse health effects of PM are due simply to structural variation affecting deposition versus fundamental differences in their effects on cardiopulmonary function.

Conclusions

In all cases evaluating pulmonary endpoints (respiratory mechanics, total respiratory resistance, responsiveness, and inflammation), the relative potency of the augmentation was coarse > fine > ultrafine = FA. Higher concentrations of coarse and fine PM were associated with greater pulmonary responses. Cardiac outcomes (i.e., changes in HR, HRV) occurred exclusively after exposures to ultrafine PM. These findings support the hypothesis that coarse and fine PM, which likely deposit in the airways, can influence lung function even in normal, control mice, while ultrafine PM can contribute to altered heart function, although the authors did not address whether these were direct effects on the heart or through alterations of autonomic regulation of the heart. These findings suggest that the PM-associated health burden is not limited to susceptible populations, such as prehypertensive patients or asthmatics but can contribute toward the development of cardiopulmonary stress in otherwise healthy, normal individuals. Although the effects observed in the normal control subjects are comparatively less severe in nature, chronic exposure to ambient PM may lead to long-lasting morbidity in these individuals and could become a serious public health concern. Future studies will be directed toward investigating the size-specific chemical constituents responsible for the differential effects observed and investigating molecular and/or biochemical pathways that link ambient PM exposure with cardiopulmonary outcomes. A strong understanding of the mechanisms responsible for these pathways in normal animal and human subjects can then be translated into studies of susceptible populations, such as asthma and heart failure/hypertension.

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Declaration of interest

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